Personalized neoantigen DNA vaccines expand tumor-specific T cells in the periphery which infiltrate the tumor in hepatocellular carcinoma

Background: Tumor neoantigens are epitopes derived from tumor-specific mutations that can be incorporated in personalized vaccines to prime T cell responses. DNA vaccines delivered with electroporation have recently shown strong CD8 and CD4 T cell responses in clinical trials. In preclinical studies, DNA-encoded neoantigen vaccines have shown induction of CD8 T cells against 50% of predicted high affinity epitopes with the ability to impact tumor growth.

Methods: Paired blood and tumor biopsy samples were collected from a patient with hepatocellular carcinoma before and after treatment with GNOS-PV02 (DNA neoantigen targeted vaccine) + plasmid IL-12 + pembrolizumab. Treatment resulted in a partial response with a decrease in tumor size of 44% by RECIST (168 mm to 94 mm). TCRbeta sequencing was performed on all 4 samples and single cell TCR and transcriptome sequencing was performed from T cells isolated from the post-treatment blood sample. Newly identified TCRs in blood and tumor after vaccination were inserted into an expression vector and used to generate engineered TCR T cells. Engineered TCR T cells were tested against the neoantigens included in the vaccine and their responses characterized by flow cytometry.

Results: We identified 67,893 new clones in PBMC after vaccination, 3 of which comprised between 0.1 to 1% of the total T cell clones. Moreover, we identified 5126 new clones in the tumor post vaccination, out of these, 3878 (75.68%) were not found within the patient's pre vaccination PBMCs and 556 (10.86%) were identified within the pre vaccination PBMC pool. Importantly, of the newly identified T cells infiltrating the tumor post vaccination, we observed high frequency TCR clones of which 44 and 7 clones were above 0.1% and 1%, respectively. The majority of the newly identified T cell clones were CD8 T cells (68.75%) with an activated phenotype. Importantly, the 6 most expanded clones in blood were identified to be activated CD8+CD69+ T cells (81.82%). Engineered TCR T cells generated encoding the TCRs of these newly identified CD8 T cells showed activation when exposed to the tumor neoantigens encoded in the neoantigen DNA vaccine GNOS-PV02.

Conclusions: GNOS-PV02, a neoantigen DNA vaccine, in combination with plasmid IL-12 and pembrolizumab resulted in expansion of newly identified T cells, primarily activated CD8, which trafficked to the tumor. These new tumor infiltrating T cells showed TCR specificity against tumor neoantigens encoded in GNOS-PV02 and may account for the observed objective decrease in tumor size.

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